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Rapid evaluation of grape phytosanitary status directly at the check point station entering the winery by using visible/near infrared spectroscopy

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The aim of this work was to investigate the applicability of vis/NIR spectroscopy for rapid assessment of grape phytosanitary status, directly at the check point at grape consignment. A device for non-contact analysis at a distance between sensor and sample of 300 mm, in the spectral range 400–1650 nm was used. Acquisitions were carried out on healthy (1235 samples, 48%) and diseased bunches (1324 samples, 52%) from different white (Chardonnay, Grillo, Inzolia, Viognier) and red (Alicante, Nero d'Avola, Syrah) varieties.

A classification analysis (Partial Least Squares – Discriminant Analysis, PLS-DA) was applied on grape spectra in order to test the classification performance of the system. The results obtained from PLS-DA models, in validation, gave a classification accuracy between 89.8% and 94.0%. Results demonstrated that the system is capable to provide useful information about wine grape phytosanitary status for a better management of the vinification process.

1. Introduction

The grape selection at the check point station entering the winery is a particularly delicate phase to obtain a qualitatively good product. Especially for big companies, such as cooperatives with a high number of partners, the vineyards spread over large spaces is characterized by soils with different (i) properties, (ii) maintenance, (iii) cultivation methods, and (iv) cultivation techniques. These differences can lead to produce grapes of the same variety with different qualities and phytosanitary status.

Currently, quantification of diseases on wine grapes is commonly performed by visually estimating the proportion of area with infection symptoms in bunches and then calculating the mean severity of the sample (Hill et al., 2014, 2013). Depending on the grapes quality, a price penalty is often enforced by wineries downgrading the grapes value up to 50% imposing a severe reduction of vine growers' income. This method is liable to assessment errors and a more objective, cost-effective and useful quantification method is needed.

Therefore, the development of an objective and timely method (without slowing down the logistic activities during the harvest, particularly hectic in large wineries) for measuring the quality and the phytosanitary status of the grape, using optical non-destructive technologies, at the check point station, could absolve wineries experts from subjective downgrade decisions and optimize the selection phase for a better management of the vinifications. The application of optical techniques could minimize contentious between the members and the winery inspectors and could improve the standardization of the grape quality and therefore of the wine.

Nowadays visible near infrared (vis/NIR) and near infrared NIR (NIRs) spectroscopy, image, and multi/hyperspectral analyses are the most applied techniques in the food sector for the quality evaluation. These optical techniques have several advantages: (i) they are non-destructive techniques, (ii) user-friendly, rapid, accurate and efficient; (iii) generate objective data that can be recorded for deferred analysis, (iv) allow a complete analysis of the lots and not just a single sample, (v) reduce the involvement of personnel in performing tedious tasks and allow the automation of various functions that would require intensive work shifts, and (vi) have reasonably affordable costs (Dale et al., 2013; Elmasry et al., 2012; Giovenzana et al., 2015; Guidetti et al., 2012).

Several efforts have been made in the past relating to optical technologies applied to analyse quality and phytosanitary status of

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wine grape.

NIR spectroscopy was used as a non-destructive technique for the assessment of changes in certain internal quality properties of wine grapes (Vitis vinifera L.) during on-vine ripening and at harvest (González-Caballero et al., 2010). NIR was applied on wine grape directly in the field to measure quality parameters and determining the optimum harvesting time (Giovenzana et al., 2014; Guidetti et al., 2010; Kemps et al., 2010), to control process like withering (Beghi et al., 2015), or, at arrival at the winery, to measure the degree of mould contamination determining payment purpose, obtaining a classification accuracy of 92% in cross-validation (Gishen et al., 2005). On grapevine leaves NIR information regarding relative vigour levels has many applications for improving management at the precision scale, such as the early detection of certain vine diseases (Hall et al., 2002) or the identification of water stress (De Bei et al., 2011).

Botrytis bunch rot in white wine grapes was estimated by Hill et al. (2014) comparing four quantification methods: digital image analysis, near-infrared (1260–1370 nm) and mid-infrared (8760–9520 nm) spectroscopy, and quantitative real-time polymerase chain reaction. Image analysis was applied to quantify the proportion of the area of individual bunches affected by Botrytis cinerea, and a significant relationship ($P < 0.05$) was found between infection severity measured by image analysis and the mean infection severity measured by human assessors for Sauvignon Blanc bunches with various degrees of infection severity ($R^2 = 0.96$). Near-infrared and mid-infrared spectroscopy were performed on homogenised berry samples to estimate botrytis bunch rot. Near-infrared gave a Ratio of Performance to Deviation (RPD) of 2.5 and MIR an RPD of 2.0. Both spectroscopic methods measured lower infection severity than that by visual estimation.

A multispectral imaging approach based on a relatively simple disease detection algorithm was applied by Oberti et al. (2014) to a dataset of grapevine leaf samples exhibiting symptoms at different levels in a view of an automatic detection of powdery mildew on grapevine leaves by image analysis.

Changes in the optical properties (450–750 nm) of white-berried grapes were investigated by Rustioni et al. (2014) with the aim of identifying the reflectance spectrum variations related to incipient browning symptoms appearance, to search for relationships with physiological processes involved in sunburning, and to emphasize chemical compositional markers of sunburning predisposition.

On grape must, the gluconic acid and glycerol content in cv. Greco di Tufo winegrapes at different rates of Botrytis cinerea infection were analyzed by Cinquanta et al. (2015) using the coupling of screen-printed amperometric biosensors with flow injection analysis to measure. Results obtained by the Authors confirmed that concentrations of both compounds are highly correlated with the rate of Botrytis cinerea infection ($R^2 = 0.98$).

A ground-based real-time remote sensing system capable to detect plant diseases automatically at an early stage of disease development and during field operations was developed by Moshou et al. (2011) for arable crops. Hyperspectral reflectance and multi-spectral imaging techniques were developed for simultaneous acquisition in the same canopy. An intelligent multi-sensor fusion decision system based on neural networks was developed to predict the presence of diseases or plant stresses, in order to treat the diseases in a spatially variable way.

Further studies are needed for the implementation of potential solutions for rapid grape evaluation in a future optical workstation at the wagons reception area of the winery. An initial testing phase of the optical systems in controlled lab-scale conditions simulating the final use of the device is therefore desirable. A future real scale application could be envisaged after setting the operative conditions to perform the measurements directly at the grape consignment check point station.

The paper describes the application of visible/near infrared spectroscopy for a rapid evaluation of phytosanitary status of grape in a view of a grape classification directly at the check point station entering the winery. The experimentation was conducted using grape bunches naturally infected with Botrytis cinerea, powdery mildew (*Erysiphe necator*) and sour rot, the major grape diseases.

2. Materials and methods

2.1. Sampling

The experimental activity took place during the harvest season 2015 (25th August/20th September 2015) at Cantine Settesoli (Menfi, AG, Italy, 37°36′14″ N, 12°58′08″ E). During the harvest period the spectral acquisitions were performed on bunches of different white grapes varieties (Vitis vinifera cv. Chardonnay, Grillo, Inzolia, Viognier) and red grapes (Vitis vinifera cv. Alicante, Nero d'Avola, Syrah), collected directly at the check point station entering the winery from the consignment wagons.

Spectral acquisitions were performed on bunches without any sample preparation in the winery laboratory, soon after samples collection, visual inspection and bunches classification. The laboratory scale measurement setup allowed to simulate in controlled environmental conditions a future operative use feasible directly at the check point station entering the winery. The laboratory setup is easily transferable to the operative scale. Measurements were carried out on healthy and diseased bunches affected by different pathologies like botrytis, powdery mildew and sour rot, for a total of 2559 analyzed samples as detailed in Table 1. Furthermore, all significant features other than disease symptoms, such as sunburn pigmentation, were recorded as possible source of false positives for automatic disease detection algorithm. Sunburned samples are healthy bunches showing optical features similar to those of diseased bunches and therefore were considered as healthy samples. Moreover, spectra were acquired on grape stalks (142 samples) and leaves (93 samples) to define a database of not-grape samples, to be automatically eliminated during spectral acquisition in a view of a future on-line application. Considering this lab-scale experimentation, stalks and leaves spectra were not considered for the data processing.

2.2. Reference disease assessment

Prior to spectral acquisition, each selected bunch underwent a pathological survey for the identification and quantification of the intensity of fungal symptoms. For each grape bunch, disease severity was estimated visually as the proportion of berries or tissue exhibiting disease symptoms when viewed from one side of the bunch with the aid of a standard area diagram (Hill et al., 2010). In particular, the diseased regions on each selected bunch were scored for disease by a plant pathologist and an oenologist from the winery, according to a 0–1 arbitrary infection-levels (IL), as follows:

IL = 0 (IL0, infection level 0), corresponding to healthy tissues and bunches with small (<2 mm in diameter) chlorotic spots with hyphae at the initial stage of development and with middle stage mycelial structures (rarely visible also with careful observation).

IL = 1 (IL1, infection level 1), extended colonies (>2 mm) with evident gray-whitish powdery appearance and brown senescent–necrotic lesions.

Bunches scored as IL0 were considered acceptable for the

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